FISEVIER

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab



Effect of degumming process on physicochemical properties of sunflower oil



Daniela Lorena Lamas*, Diana Teresita Constenla, Daniela Raab

PLAPIQUI-UNS-CONICET, Pilot Plant of Chemical Engineering, National University of the South, National Scientific and Technical Research Council, La Carrindanga Km 7, Bahía Blanca, 8000 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form
14 March 2016
Accepted 15 March 2016
Available online 16 March 2016

Keywords:
Enzymatic degumming
Water degumming
Sunflower oil
Efficiency
Phospholipase enzymes
Physicochemical properties

ABSTRACT

The effect of different degumming processes on physicochemical properties of crude sunflower oil was studied by means phosphorous content, acid value, peroxide value, phospholipids, iron, moisture, unsaponifiable matter, viscosity, density and color value. Three different degumming processes were evaluated: phospholipase A1 degumming process, phospholipase A2 degumming process and water degumming process. The enzymatic degumming trials were performed at 50 °C, pH 5 and an enzyme dosage of 200 U/kg of oil during 180 min with both enzymes. The water degummed treatment was carried out at the same time with 3% water/oil ratio, and 65 °C of temperature. The phosphorus content decreased from 544.51 to 3.02 mg/kg and 5.81 mg/kg using phospholipase A1 and A2 respectively. These results indicated the high efficiency of the treatments to achieve good quality oil suitable for physical refining. The residual phosphorus content value obtained for water degummed treatment suggests that it was not enough for physical refining process. Phospholipids content showed a drastic decrease with the enzymatic degumming process. The iron content was reduced with all degumming processes, being more significantly after the enzymatic treatment. The unsaponifiable matter decreased in all treated samples compared with crude sunflower oil. Impurities that enhance the viscosity of oil and some pigments were removed during the process, being more noticeably in the enzymatic treatment. The results obtained revealed that the enzyme-mediated degumming oils had better physicochemical parameters than the crude and water degummed oils.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Crude sunflower oil obtained by oilseed processing has to be refined before the consumption in order to remove undesirable compounds. The most important of these minor substances are free fatty acids, color pigments, phospholipids, metals and waxes. The objective of refining is to eliminate these impurities with the least possible effect on desirable components present in the crude vegetable oils in order to obtain an odorless, bland and oxidative stable refined vegetable oil that is acceptable to consumers (Medina-Juarez et al., 2000). Presence of compounds such as odiferous volatiles, pigments, waxes and metal traces, affect negatively the taste, smell, appearance and storage stability of the refined oil. They must be removed to yield a stable product with a bland or pleasant taste (Aluyor et al., 2009). The presence of phospholipids can cause the oil discoloration, serve as a precursor of off-flavors and contribute to the losses of neutral lipids during

E-mail address: dlamas@plapiqui.edu.ar (D.L. Lamas).

neutralization (Jiang et al., 2015). In addition, phospholipids are naturally occurring emulsifiers, which bind oil molecules together leading to increased viscosity, and refining and/or flow losses (Iwuoha et al., 1996). In the food industry, viscosity is one of the most important parameters required in the design of technological process. On the other side, viscosity is also an important factor that determines the overall quality and stability of a food system (Abramovic and Klofutar, 1998). Therefore, the removal of phospholipids is essential for the production of high-quality finished oil (Subramanian et al., 1999). Degumming is an important step in the refining process of vegetables oils, and it removes phospholipids and mucilaginous gums. Traditional degumming that include water, super, total, acid degumming and ultrafiltration processes cannot guarantee the low phosphorus levels that are required for physical refining (Jiang et al., 2015). These techniques are not suitable for oils with high levels of non-hydratable phospholipids (Zufarov et al., 2008). Enzyme-mediated degumming is a unique process quite distinct from the well-known acid degumming variations, since both hydratable and nonhydratable phospholipids present in the oil are hydrolysed to the corresponding

^{*} Corresponding author.

lysophospholipids (Clausen, 2001).

Currently, the most commonly utilized phospholipases in enzymatic degumming are phospholipase A1 (PLA1) and phospholipase A2 (PLA2), which remove the fatty acid from positions 1 and 2 with respect to glycerol, respectively (Galhardo et al., 2010). Other enzymes are commercially available for vegetable oils processing, such as phospholipase B (PLB), phospholipase C (PLC) and the lipid acyltransferase (LAT). PLB eliminates both fatty acids from the glycerol group (Jiang et al., 2011), PLC catalyses the hydrolysis of phosphate-glycerol bond in phosphatidylcholine and phosphatidylethanolamine and LAT, transfers a fatty acid to a sterol present in the oil in order to convert it into a sterol ester (Dijkstra, 2011).

There are several studies about the effect of enzymatic degumming on the phosphorus content (Jiang et al., 2011; Manjula et al., 2011), the quantitative and qualitative analysis of phospholipids (Sampaio et al., 2015) and the acid value achieved (Jahani et al., 2008). However, just few studies have been undertaken to determine the changes in physical and chemical characteristics of sunflower oil during the enzymatic degumming process. The aim of this work was to evaluate the effect of the different degumming process on physical and chemical parameters of crude sunflower oil.

2. Materials and methods

2.1. Materials

Molinos Río de la Plata (Rosario, Argentina) supplied the crude sunflower oil extracted by hexane utilized in this work (Table 1). The oil was stored in tightly sealed amber container at 5 °C for further use. All reagents were of analytical reagent grade. Citrate buffer (pH 5.0) was prepared by mixing citric acid solution (0.1 M) and sodium hydroxide solution (0.1 M), both made with double-distilled water, for stock solutions require to yield a desired pH value in each case. Lecitase® Ultra an acidic PLA1 (EC 3.1.1.32) from Thermomyces lanuginosus expressed in Aspergillus oryzae was acquired from Novozymes (Bagsvaerd, Denmark). This enzyme

 Table 1

 Characterization of crude hexane extracted sunflower oil.

Parameter	Crude Oil	
Phosphorous content (mg/kg)		
Acid value (mg KOH/g)	544.510 ± 19.830	
, , , , , , , , , , , , , , , , , , , ,	2.290 ± 0.050	
Moisture (g/100 g)	0.075 ± 0.010	
Peroxide value, PV (meq/kg)	3.660 + 0.160	
Unsaponifiable matter (g/kg)	_	
Phospholipids (g/100 g)	8.760 ± 0.500	
Iron content (mg/kg)	$\textbf{0.944} \pm \textbf{0.110}$	
, 5, 5,	9.410 ± 0.410	
Viscosity (mPa*s)	46.710 ± 2.120	
Density (kg/m 3)	921.800 + 3.020	
Color	321.000 <u>+</u> 3.020	
L*	58.860 ± 2.800	
a*	5.850 + 0.370	
b *	_	
	33.800 ± 2.600	

Results are mean values \pm standard deviation analyzed by triplicate.

exhibits PLA1 activity at pH values from 4.5 to 6 at 50 °C according to the manufacture's instruction. MAXAPAL® A2, a liquid PLA2 (E. C. 3.1.1.4) obtained by submerged culture of a selected strain of *Aspergillus niger* was provided by DSM Food Specialties (Netherlands). This enzyme is active in the pH range 5.0–9.5 according to the manufacture's instruction.

2.2. Oil degumming assay system

The assay system consisted of a jacketed reactor fitted with lid, a propeller and a thermometer. The reactor was connected to a water bath with water pump and flexible tube.

For enzymatic degumming, the most influential factors include temperature, pH, buffer/substrate ratio and enzyme dosage. Their values were set based on preliminary assays and literature data (Lamas et al., 2014; Yu et al., 2014). Crude sunflower oil (approximately 1000 g) was loaded in the reactor, which was kept at 50 °C of temperature. The assay was performed at pH 5 by the addition of 2% buffer/substrate ratio. Followed by the addition of 200 U/kg of oil enzyme solution for both enzymes, the mixture was stirred with automatic mixer to provide a safe large surface area through emulsification. The process was performed during 180 min. The stop reaction was carried out during 30 min at 100 °C to inactivate the enzyme. To recover oil and water phases a centrifuged step was applied (10 min at 2400xg).

For water degumming, the experiment was carried out in the same reactor. When the heating temperature of sunflower oil $(1000\,\mathrm{g})$ reached 65 °C, 3% of distilled water was added. The mixture allowed under stirring during the process. Finally, the oil was centrifuged (10 min at 2400 xg) to separate the gums to yield water degummed oil.

2.3. Analytical methodology

2.3.1. Efficiency

To evaluate the efficiency of enzymatic degumming processes, the phosphorus content determination in crude and degummed oil samples was used. The phosphorous content was determined by ashing the sample in the presence of zinc oxide, followed by the spectrometric measurement of phosphorous as a blue phosphomolybdic acid complex according to AOCS Official Method Ca 12-55 (Firestone, 2009). The absorbance was measured at 650 nm by using a Shimadzu 160 UV–vis spectrophotometer equipped with a computer-assisted system for data acquisition (Shimadzu 160 Japan). The efficiency of each degumming process was estimated based on its ability to reduce the phosphorus content using the following equation:

$$Efficiency = (Pi - Pr)/Pi$$
 (1)

where Pi is the total phosphorus content of the crude oil (mg/kg) and Pr is the residual phosphorus content in degummed oils (mg/kg).

2.3.2. Physicochemical indexes

Acidity was measured by titration with a standardized ethanolic solution of potassium hydroxide and phenolphthalein as indicator. The analysis was determined according to AOCS Official Method Cd 3d-63 (Firestone, 2009). An acetic acid-chloroform AOCS Official Method Cd 8-53 (Firestone, 2009) for peroxide value was employed to measure peroxides and other similar compounds that oxidize potassium iodide as primary oxidation products.

Quantitative determination of phospholipids was carried out by enrichment using diol solid phase extraction cartridges (J.T. Baker Inc., Phillipsburg, NJ) and subsequent analysis by high-performance liquid chromatography according to AOCS Official Method

Ja 7b-91 (Firestone, 2009). Waters HPLC e2695 Alliance systems with a Waters 2998 Photodiode Array Detector (PDA) set at 206 nm and an EC 250/4.6 Nucleosil 50-5 de Macherey-Nagel column were used. The mobile phase used was n-hexane/2-propanol/acetate buffer pH 4.2 (8:8:1, v/v/v). The flow rate was set at 2 mL/min.

The determination of iron content was performed by atomic absorption spectrophotometry flame. The oil sample was treated with open flame and then it was carried to ashes in a muffle furnace at 550 °C. The ashes were taken up with concentrated hydrochloric acid and hot distilled water. The determination was performed using an Atomic Absorption Spectrometer GBC 902AA. The measurement was performed by external calibration using a certified standard pattern. Moisture and volatile matter was determined using AOCS Official Method Ja 2a-46 (Firestone, 2009). Unsaponifiable matter was evaluated by AOCS Official Method Ca 6a-40 (Firestone, 2009).

To perform a color experiment of the oil samples, the HunterLab UltraScan XE tristimulus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) was used. An aliquot of each sample was taken to fill a glass cuboid cell (10 mm thickness), and the total color transmitted through the sample was measured at 10° observer angle with D65 illuminant. Results were expressed as the Hunter Lab scale parameters L^* (lightness: 0=black, 100=white), a^* [greenness (-), redness (+)] and b^* [blueness (-), yellowness (+)].

Measurements of the viscosity at 25 °C were performed with an Anton Paar Physica Rheometer MCR 301 with a cell P-PTD 200 to concentric cylinders. A rotating inner cylinder B-CC27/148/E and an outer cylinder C-CC27/55 were used. Density was determined at 25 °C using a pycnometer according to the AOCS Official Method Cc 10c-95 (Firestone, 2009).

2.4. Statistical analysis

All degummed experiments were performed in duplicate. For statistical evaluations, all analytical data generated from each group of degummed oils were subjected to analyses in triplicated. The crude oil was evaluated in triplicate as well. Differences among the means were compared using the Duncan test, being statistically different at significance level of 5%.

3. Results and discussion

3.1. Efficiency and oil yield of degummed process

The phosphorus content of crude sunflower oil was 544.51 mg/kg. This result is consistent with the value detected in a previous

study using other set of hexane extracted sunflower oil (Lamas et al., 2014). Enzymatic degumming with PLA1 and PLA2 reduced the phosphorus content to 3.02 and 5.81 mg/kg, respectively after 180 min of reaction. The reduction of phosphorus below to 10 mg/kg in the degummed oil is the prerequisite of physical refining (Jahani et al., 2008). The monitoring of the process was conducted in order to meet the demands of physical industrial refining. As showed in Fig. 1, the phosphorus content decreased by increasing reaction time using both enzymes. However, the least of the reaction time that oil was degummed to less than 10 mg/kg of phosphorus was 60 min in the treatment using PLA1, while PLA2 needed more than 120 min to achieve the physical refining requirements.

These results are close with those reported by previous studies using the same enzymes. In the case of Lecitase Novo (PLA1 from *Fusarium oxysporum*), the phosphorus content was reduced to 5 mg/kg in rapeseed oil degumming (Clausen, 2001). Jahani et al. (2008) reported that in the case of rice brain oil, the optimal operating conditions were reaction time of 4.07 h, enzyme dosage of 50 mg/kg and added water of 1.5%. At this optimal point, phosphorous content of degummed oil was found to be 8.86 mg/kg. The phosphorus content of the crude soybean oil was reduced to less than 10 mg/kg after 120 min time using 30 mg/kg of PLA1 enzyme dosage (Sampaio et al., 2015). In rapeseed oil samples, the residual phosphorus could be reduced below 10 mg/kg by the use of PLA1 without acid pretreatment (Jiang et al., 2015).

A free pancreatic mammal PLA2 required 5 h to reach the phosphorous content of 10 mg/kg in soybean oil (Yu et al., 2014). The PLA2 from *Streptomyces violaceoruber* reduced the phosphorus content from 261.77 mg/kg to 20.74 mg/kg during degumming treatment, being not enough for physical refining (Liu et al., 2015). Therefore, PLA1 seems to be more effective than PLA2 in different types of oil. The PLA Lecitase[®] Ultra has been reported as a microbial enzyme with PLA1 and PLA2 activity (Mansfeld, 2009), so this enzyme could be extracting the fatty acid from position 1 and 2 with respect to glycerol. Also, fatty acids have a preference for the 1,3-positions, which is why 1,2-diglycerides produced by A1-catalysed hydrolysis readily isomerise to 1,3-diglycerides form and can then be hydrolysed again to form glycerol phosphate esters (Dijkstra, 2011).

The residual phosphorus content after 180 min of water degumming treatment was 63.21 mg/kg. This result could be due to the non-hydratable phospholipids remained after water treatment (Yang et al., 2008) and suggested that water degumming is not effectively in order to degum sunflower oil for physical refining. It has been reported previously that applying water degumming treatment on sunflower oils, the decreasing of phosphorus content was not enough for use in physical refining (Zufarov et al., 2008).

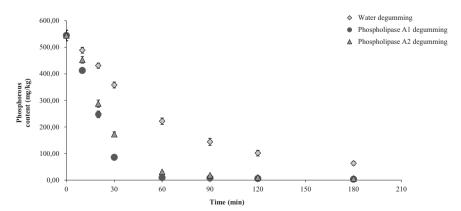


Fig. 1. Change of phosphorous content by the water and enzymatic degumming process. Results are means values (n=3) by duplicate degumming assays. Error bars represent the standard deviations of mean values.

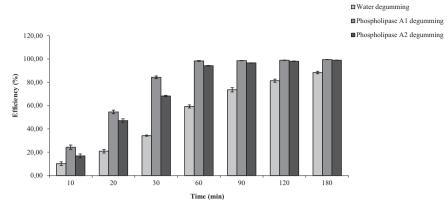


Fig. 2. Efficiency of the water and enzymatic degumming process. Results are means values (n=3) by duplicate degumming assays. Error bars represent the standard deviations of mean values. Different letters indicate significant differences (P < 0.05).

In the case of rice brain oil, water degumming process reduced the phosphorus content to 128 mg/kg (Manjula et al., 2011). A similar behaviour was observed in rapeseed, camelia and soybean oil (Jiang et al., 2015).

Compared to the water-degumming process higher degumming efficiency was observed by using enzymes treatments (Fig. 2). The efficiency by water degummed process was 88.4%, whereas a higher percentage around 99% was achieved for enzymatic degumming using both enzymes. However, the results of PLA1 and PLA2 had time difference in their efficiencies. This could be due to the behaviour and requirements of the enzymes. The PLA1 preferentially hydrolyses the phosphatides that are present in the oil phase whereas the PLA2 prefers the phospholipids present in the aqueous phase (Dijkstra, 2011). Thus, PLA2 is slower to cause a reduction in the phosphorous content and to achieve a high efficiency.

During the water degumming process hydratable phospholipids absorb water to agglomerate into a gum phase, which is separated with centrifuging step (Zufarov et al., 2008). However, the water degumming does not remove nonhydratable salts of phosphatidic acids (Dijkstra, 2011). Therefore, the phosphorous content determined at the oily phase represented the phospholipids no catalysed. The results have clearly shown that the phosphorous content and efficiency achieved were very different depending on

the degumming process applied on the oil samples.

3.2. Physicochemical properties

Results of the effects of degumming process on the chemical and physical characteristics of edible sunflower oil are shown in Table 2. The acid value of crude sunflower oil was 2.29 expressed as mg KOH/g oil. This result is in accordance with those reported by Tasan et al. (2011) for the same type of crude oils. Abitogun and Oshodi (2010) found that acid value content was 2.78 mg KOH/g oil in the crude sunflower oil obtained by hexane extraction. Treatment of crude oil with enzymes could result in a significant increase in the acid value in the oil. It is well known that the free fatty acid from position 1 with respect to glycerol could be removed from a phospholipid molecule in the enzymatic degumming process by PLA1. Thus, hydrolysis of ester bonds produced the liberation of free fatty acids. The PLA2 removes the fatty acid from position 2 with respect to glycerol. Therefore, the increase of free fatty acids detected was the consequence of the hydrolysis of phospholipids. Yang et al. (2006) reported that the degumming process using PLA1 in rapeseed and soybean oils caused a slight increase of acid value in the degummed oils. Similarly, in the case of rice bran oil was reported that Lecitase[®] Ultra increased the free fatty acids content (Jahani et al., 2008).

 Table 2

 Physicochemical characteristics obtained for water and enzymatic degummed oils.

Parameter	Sample				
	Water degummed oil	PLA1 degummed oil	PLA2 degummed oil		
Phosphorous content (mg/kg)	ca ata y c agah	2 222 . 2 222	5.040 . 0.4003		
Acidity (mg KOH/g)	$63.210 \pm 6.990^{\mathrm{b}}$	$3.020 \pm 0.200^{\mathrm{a}}$	5.810 ± 0.400^{a}		
Actuaty (mg KOn/g)	2.350 ± 0.100^a	3.620 ± 0.280^{b}	3.340 ± 0.170^{b}		
Moisture (g/100 g)	0.109 ± 0.021^{b}	0.102 ± 0.010^a	0.106 ± 0.016^{a}		
Peroxide value, PV (meq/kg)	3.900 ± 0.090^a	3.940 ± 0.090^a	3.810 ± 0.080^a		
Unsaponifiable matter (g/kg)	6.060 ± 0.100^a	7.480 ± 0.490^{b}	7.450 ± 0.460^{b}		
Phospholipids (g/100 g)	0.123 ± 0.018^{b}	0.047 ± 0.001^a	0.054 ± 0.002^a		
Iron content (mg/kg)	6.410 ± 0.420^{c}	2.260 ± 0.170^{a}	4.150 ± 0.160^{b}		
Viscosity (mPa*s)	45.620 ± 1.100^{b}	42.150 ± 2.140^a	42.270 ± 1.780^a		
Density (kg/m³)	920.900 ± 4.200^b	915.200 ± 3.000^a	913.900 ± 2.900^{a}		

The release of free fatty acids is responsible for the development of the undesirable rancid flavor (hydrolytic rancidity). Therefore, any increase in the acidity of the oil must be absolutely avoided. In the present case, the obtained-acid values not represent drawback for its reduction during refining process. A slightly higher value, although not significantly different, was found for the water degummed oil with respect to the crude oil. This increased may be caused by heat and higher moisture content achieved during the process indicating high sensitivity to hydrolytic damage (Vidrih et al., 2010). The moisture content of the degummed oils was greater than that of crude oil. This increasing could be due to the addition of the buffer and aqueous solution necessary for the degummed treatments (Lamas et al., 2014).

The peroxide value, as a measure of primary oxidation products, was 3.66 meq/kg in the crude sunflower oil. For proper handling, Gupta (2003) suggested maintain a peroxide value of less than 4 in the crude oil during storage. For enzymatic degumming, the peroxide value was found to be 3.94 meq/kg using PLA1 and 3.81 meq/kg with the PLA2 treatment. A slight increment in the all degummed samples was obtained. The high peroxide value of the degummed oil might be due to oxidation caused by moisture achieved with the degumming process.

The unsaponifiable matter contains different compounds as sterols, alcohols, tocopherols, phenols and hydrocarbons. The unsaponifiable matter (Table 2), decreased by 14% in enzyme-degummed treated samples compared with crude sunflower oil. The result obtained after water degummed showed a higher reduction. These decreases could be due to removal of some minor compounds during the degumming processes.

The sunflower oil contains phospholipids, mucilage compounds and resins as gummy substances, which impart color turbidity and odor to the oil. Phospholipids are the major component of gum, which increases refining losses, create foaming problem and generate more color. To estimate the phospholipid contents in oils, a factor of 25 is usually applied, to convert the percentage of total phosphorous to the equivalent content of phosphatides (Galhardo et al., 2010). However, this factor overestimates the phospholipids and lower factors have been proposed (Carelli et al., 2002). The differences could be attributed to phosphorus from sources other than phospholipids such as sand and meal residues, including inorganic phosphorous that is also determined by the spectrophotometric method, and to the presence of minor phospholipids not quantified by chromatographic analysis. The contents of phospholipids in degummed oils determined by the chromatographic method are shown in Table 2. The phospholipids content of crude sunflower oil was strongly reduced by the enzymatic degumming process being 95.05% and 94.28% with PLA1 and PLA2 respectively. This should be due to the hydrolysed capacity of the enzymes. The water degumming process also achieved a significant reduction in the phospholipids content. However, the residual phospholipids content is noticeably higher than the result obtained by the enzymatic treatment. The residual phospholipids content could be due to the non-hydratable phospholipids remained after water treatment (Yang et al., 2008).

Iron metal is one of the most common contaminants (Lamas et al., 2014). Its presence in crude oil is due to natural iron present in oilseeds, mainly bounded to phospholipids and other minor compounds, and possible contamination from metallic equipment (Brevedan et al., 2000). A great difference between crude oil and treated oils iron content was determined. When PLA1 was used for the oil degumming, the iron content of the crude oil decreased from 9.41 to 2.26 mg/kg. As well, the iron content of crude sunflower oil was reduced to 4.15 mg/kg after degumming using the PLA2 enzyme. The results obtained also indicated that water degumming process reduced the content of this metal. This may indicate that a significant iron concentration was complexed with

phospholipids (Brevedan et al., 2000).

The data obtained for viscosity showed that crude sunflower oil exhibited the highest resistance to flow with a viscosity value of $48.63 \pm 2.65 \, \text{mPa*s}$, while enzymatic degummed oil samples showed the least data with no significant differences between them. The water treatment exhibited a least reduction, but it was significant statistically. These results implied that the degrading impurities, that enhance the viscosity of oil, have been removed during the course of the degumming treatment. Iwuoha et al. (1996) reported similar tendencies in palm, palm kernel and groundnut oils using different reagents for degumming.

The process of degumming of sunflower oil had little effect on density. The density of enzymatic degummed oil sample was lower than the value for unrefined oil. These reductions may be due to the removal of some compounds from the oil by enzymatic treatment. Similar observations were recorded by Tyagi et al. (2012) and Agrawal (2013) in rice bran oil. The water degummed process not showed a significant change on density parameter value obtained.

Crude vegetable oils contain various natural color substances (Tasan et al., 2011). The changes in the lightness (L^* value), redness (a^* value) and yellowness (b^* value) over degumming treatment are shown in Table 3. Lightness increased considerably during degumming process. The L^* value results obtained show that PLA1 degumming gave the greatest color increase while water degummed agent gave the least increment. This measure indicates the reduction of the main compounds that contribute to color of oils. The L^* parameter color value obtained for the all oil samples indicates that crude oil is darker.

The most significant changes are observed in a^* values, which are related with the tonality of color, changing from redness (a^*) to greenness $(-a^*)$ along the treatment. The change was more significant in the enzyme treated samples. The b^* value of enzymatic degummed with PLA2 agent was the highest. All the samples tested positive for this parameter, indicating a tendency to yellow tones.

The results obtained are close with previous studies in other type of oils. Tyagi et al. (2012) reported that the phosphoric acid treatment resulted in marked reduction in color of rice bran oil. Others studies in rice bran oil, reported that the color was effectively improved by the removal of phospholipids by the degumming process using water and phosphoric acid mixture (Agrawal, 2013). Cleenewerck and Dijkstra (1992) revealed that darkening of the oil was related to its iron content. A correlation between iron content and the color parameters was detected in the present work, suggesting that high contents of this metal adversely affect the color of the oil. These observations are suggestive that viscouscausing phospholipids, trace metals and dark pigments have been essentially removed. This could mean a benefit for a period after process.

4. Conclusion

The physicochemical characteristics of the oil improve upon

Table 3 Values obtained by the Hunter L^* , a^* , b^* color scale for degummed oils samples.

Sample	L*	a*	b*
Water degummed oil PLA1 degummed oil PLA2 degummed oil	$62.02 \pm 3.59^{a} \\ 76.53 \pm 3.77^{c} \\ 71.15 \pm 2.22^{b}$	$\begin{aligned} &-1.72 \pm 0.11^a \\ &-2.01 \pm 0.18^b \\ &-1.80 \pm 0.23^{ab} \end{aligned}$	$\begin{array}{c} 35.13 \pm 1.62^a \\ 43.01 \pm 2.53^b \\ 42.64 \pm 2.26^b \end{array}$

Results are mean values \pm standard deviation (n=3) of duplicate degumming assays.

Different letters in the same column indicate significant differences (P < 0.05).

treatment with water and enzymes. The phosphorus content achieved during the enzymatic degumming process was lower than 10 mg/kg using both enzymes. During the time studied, the water degumming process it was not enough to meet the physical refining requirements. The acid value was in accordance with the enzymes behaviour. The PLA2 activity caused a minor increment in acid and peroxide value with respect to crude oil. So, it is suggested implement this enzyme for degumming of less-stable oils. Iron was better extracted by PLA1 than by PLA2. Therefore, it is recommended the use of Lecitase[®] Ultra enzyme when the oil to be treated has high iron metal content. The enzymatic degumming process reduced the phospholipids content in crude sunflower oil noticeably. The content of unsaponifiable matter in the degummed oils was lower compared to crude oils. Impurities that enhance the viscosity of oil and some pigments were removed during all the processes analyzed. The general enhance in quality and composition of sunflower oil by the degumming processes could be a benefit for a period after process.

Acknowledgements

This work was supported by Pilot Plant of Chemical Engineering, National University of the South and National Scientific and Technical Research Council (Grant no. PGI 24/M 116-UNS). Authors would like to thank Molinos Río de La Plata for provide oil samples. We are also grateful to DSM Food Specialties Netherlands and Novozymes Denmark for donating the enzymes.

References

- Abitogun, A.S., Oshodi, A.A., 2010. Effects of degumming and bleaching on physicochemical properties of crude sunflower oil. J. Environ. Agr. Food Chem. 9, 1145–1151
- Abramovic, H., Klofutar, C., 1998. The temperature dependence of dynamic viscosity for some vegetable oils. Acta Chim. Slov. 45, 69–77.
- Agrawal, A., 2013. Comparative study on oil products of rice bran. J. Glob. Res. Comput. Sci. 4, 22–24.
- Aluyor, E., Aluyor, P., Ozigagu, C., 2009. Effect of refining on the quality and composition of groundnut oil. Afr. J. Food Sci. 3, 201–205.
- Brevedan, M.I.V., Carelli, A.A., Crapiste, G.H., 2000. Changes in composition and quality of sunflower oils during extraction and degumming. Grasas Aceit. 51, 417–423
- Carelli, A.A., Ceci, L.N., Crapiste, G.H., 2002. Phosphorous-to-phospholipid conversion factors for crude and degummed sunflower oils. J. Am. Oil Chem. Soc. 79, 1177–1180.
- Clausen, K., 2001. Enzymatic oil-degumming by a novel microbial phospholipase. Eur. J. Lipid Sci. Technol. 103, 333–340.

- Cleenewerck, B., Dijkstra, A.J., 1992. The total degumming process- theory and industrial application in refining and hydrogenation. Fat. Sci. Technol. 94, 217–222
- Dijkstra, A.J., 2011. Enzymatic degumming. Lipid Technol. 23, 36-38.
- Firestone, D., 2009. Official Methods and Recommended Practices of American Oil Chemist's Societyl, sixth ed. AOCS Press, Champaign, Illinois, USA.
- Galhardo, F., Dayton, C., Autino, H., Odone, M., Imoda, V., Lascano, A., 2010. Enzymatic degumming of vegetable oils. Comparison of different process options. A&G 79, 204–212.
- Gupta, M.K., 2003. Fundamental quality control in vegetable oil refining. Oil Mill. Gazet. 108, 6–9.
- Iwuoha, C.I., Ubbaonu, C.N., Ugwo, R.C., Okereke, N.U., 1996. Chemical and physical characteristics of palm, palm kernel and groundnut oils as affected by degumming. Food Chem. 55, 29–34.
- Jahani, M., Alizadeh, M., Pirozifard, M., Qudsevali, A., 2008. Optimization of enzymatic degumming process for rice brain oil using response surface methodology. LWT Food Sci. Technol. 41, 1892–1898.
- Jiang, F., Wang, J., Ju, L., Kaleem, I., Dai, D., Li, C., 2011. Optimization of degumming process for soybean oil by phospholipase B. J. Chem. Technol. Biotechnol. 86, 1081–1087.
- Jiang, X., Chang, M., Jin, Q., Wang, X., 2015. Application of phospholipase A1 and phospholipase C in the degumming process of different kinds of crude oils. Process Biochem. 50, 432–437.
- Lamas, D.L., Crapiste, G.H., Constenla, D.T., 2014. Change in quality and composition of sunflower oil during enzymatic degumming process. LWT Food Sci. Technol. 58, 71–76.
- Liu, A., Yu, X.W., Sha, C., Xu, Y., 2015. Streptomyces violaceoruber phospholipase A2: expression in *Pichia pastoris*, properties, and application in oil degumming. Appl. Biochem. Biotechnol. 175, 3195–3206.
- Manjula, S., Jose, A., Divakar, S., Subramanian, R., 2011. Degumming rice bran oil using phospholipase-A1. Eur. J. Lipid Sci. Technol. 113, 658–664.
- Mansfeld, J., 2009. Plant phospholipases A2: perspectives on biotechnological applications. Biotechnol. Lett. 31, 1373–1380.
- Medina-Juarez, L.A., Gamez, M.N., Ortega, G.J., Noriega, R.J.A., Angulo, G.O., 2000. Trans fatty acid composition and tocopherol content in vegetable oils produced in Mexico. J. AM. Oil Chem. Soc. 77, 721–724.
- Sampaio, K.A., Żyaykina, N., Wozniak, B., Tsukamoto, J., De Greyt, W., Stevens, C.V., 2015. Enzymatic degumming: Degumming efficiency versus yield increase. Eur. J. Lipid Sci. Technol. 117, 81–86.
- Subramanian, R., Nakajima, M., Yasui, A., Nabetani, H., Kimura, T., Maekawa, T., 1999. Evaluation of surfactant-aided degumming of vegetable oils by membrane technology. J. AM. Oil Chem. Soc. 76, 1247–1253.
- Tasan, M., Gecgel, U., Demirci, M., 2011. Effects of storage and industrial oilseed extraction methods on the quality and stability characteristics of crude sunflower oil (*Helianthus annuus L.*). Grasas Aceites 62, 389–398.
- Tyagi, K., Ansari, M.A., Tyagi, S., Tyagi, A., 2012. A novel process for physically refining rice bran oil through degumming. Adv. Appl. Sci. Res. 3, 1435–1439.
- Vidrih, R., Vidakovic, S., Abramovic, H., 2010. Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined vegetable edible oils. Czech J. Food Sci. 28, 376–384.
- Yang, J.G., Wang, Y., Yang, B., Mainda, G., Guo, Y., 2006. Degumming of vegetable oil by a new microbial lipase. Food Technol. Biotechnol. 44, 101–104.
- Yang, M., Zhou, X., Jin, Y., 2008. Non-hydrated phosphatide and its quantitative examination. Chin. J. Health Lab. Technol. 18, 71–72.
- Yu, D., Ma, Y., Jiang, L., Walid, E., He, S., He, Y., Xiaoyu, Z., Zhang, J., Hu, L., 2014. Stability of soybean oil degumming using immobilized phospholipase A (2). J. Oleo Sci. 63, 25–30.
- Zufarov, O., Schmidt, S., Sekretar, S., 2008. Degumming of rapeseed and sunflower oils. Acta Chim. Slov. 1, 321–328.